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Food intake and energy expenditure in growing cats with and without a predisposition to overweight

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Summary
Overweight and obesity are multifactorial diseases caused by an imbalance in energy metabolism. An underlying genetic predisposition is often a factor in these conditions. In the cat breeding colony of the Institute of Animal Nutrition at the Vetsuisse Faculty, University of Zurich, a segregating overweight phenotype with a genetic contribution was observed. From this breeding colony, 26 kittens were followed from birth up to 8 months of age. During this time, food intake was measured using an automatic feeding station, and energy expenditure was investigated using indirect calorimetry at the ages of 4 and 6 months. Dual energy X-ray absorptiometry (DEXA) was performed and blood glucose, leptin, and insulin were measured at the ages of 4, 6 and 8 months. The kittens were also weighed daily for the first 2 weeks of life, every second day until weaning and once per week until 8 months of age. The body condition score (BCS) was evaluated monthly between 2 and 8 months of age. The main finding of the present study is that a predisposition to overweight is connected to a higher food intake early in life, with no significant alterations in energy expenditure. The leptin blood levels were related to body fat percentage, and insulin sensitivity did not seem to be affected.

Keywords: leptin, insulin sensitivity, Dual energy X-ray absorptiometry, indirect calorimetry

Zusammenfassung
Übergewicht und Obesitas sind multifaktorielle Ekrankungen, verursacht durch ein Ungleichgewicht in der Energiebilanz. Eine zu Grunde liegende genetische Prädisposition ist häufig ein Faktor der an diesen Zuständen beteiligt ist. Bei den Zuchtkatzen des Insituts für Tierernährung der Universität Zürich wurde ein segregierender Phänotyp mit einem genetischen Hintergrund, der zu Übergewicht prädisponiert, beobachtet. Von dieser Zuchtfamilie, wurden 26 Katzenwelpen von der Geburt bis zu einem Alter von 8 Monaten erforscht. Im Alter von 4 und 6 Monaten wurde die Futteraufnhame, mittels einer automatisierten Futterstation, und der Energieumsatz, mittels indirekter Kalorimetrie, gemessen. Mit 4, 6 und 8 Monate wurde eine Dual- Energie-Röntgen-Absorptiometrie (DEXA) durchgeführt und die Blutkonzentrationen von Glukose, Leptin und Insulin gemessen. Die Welpen wurden in den ersten 2 Lebenswochen täglich gewogen, danach jeden zweiten Tag bis zum Absetzen und anschliessend wöchentlich bis zum 8. Lebensmonat. Die Körperkondition (Body Condition Score, BCS) wurde monatlich ab einem Alter von 2 Monaten bis zum 8. Lebensmonat evaluiert. Hauptbefund dieser Studie ist, dass die Prädisposition zu Übergewicht bereits im frühen Lebensalter verbunden ist mit einer erhöhten Futteraufnahme, ohne Veränderungen im Energieumsatz. Die Leptinkonzentration korrelierte im Blut mit dem Körperfettgehalt, die Insulinsensitivität hingegen war nicht beeinträchtigt.

Schlüsselwörter: Leptin, Insulinsensitivität, Dual-Röntgen-Absorptiometrie, indirekte Kalorimetrie

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Food intake and energy expenditure in growing cats with and without a predisposition to overweight

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Keywords: leptin, insulin sensitivity, Dual energy X-ray absorptiometry, indirect calorimetry

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Summary
Overweight and obesity are multifactorial diseases caused by an imbalance in energy metabolism. An underlying genetic predisposition is often a factor in these conditions. In the cat breeding family of the Institute of Animal Nutrition at the Vetsuisse Faculty, University of Zurich, a segregating overweight phenotype with a genetic contribution was observed. From this breeding family, 26 kittens were followed from birth up to 8 months of age. During this time, food intake was measured using an automatic feeding station, and energy expenditure was investigated using indirect calorimetry at the ages of 4 and 6 months. Dual energy X-ray absorptiometry (DEXA) was performed and blood glucose, leptin, and insulin were measured at the ages of 4, 6 and 8 months. The kittens were also weighed daily for the first 2 weeks of life, every second day until weaning and once per week until 8 months of age. The body condition score (BCS) was evaluated monthly between 2 and 8 months of age. The main finding of the present study is that a predisposition to overweight is connected to a higher food intake early in life, with no significant alterations in energy expenditure. The leptin blood levels were related to body fat percentage, and insulin sensitivity did not seem to be affected.

Introduction
Obesity has been identified as the second most common health problem in domestic cats in developed countries (Cave et al. 2012). The prevalence of feline obesity between 2000 and 2008 ranged from 6% to 52% in Western Europe (Russell et al. 2000; Colliard et al. 2009). Feline overweight and obesity are often related to neutering, low activity levels (indoor cats), the availability of highly palatable food, and a genetic component (German 2006; Kienzle and Bergler 2006; Colliard et al. 2009). In the study by Haring et al. (2012) conducted with cats from the breeding family of the Institute of Animal Nutrition of the Vetsuisse Faculty (IAN) at the University of Zurich, overweight was observed in juvenile eight-months-old cats. In small children, overweight and obesity are caused by genetic, behavioral and environmental factors (Xu and Xue 2016). Overweight is the result of an imbalance between energy intake and total energy expenditure and is caused by a higher food intake and/or a lower energy expenditure (Fettman et al. 1997). Food intake and energy expenditure are regulated in the hypothalamus and in the caudal brainstem by different adipokines (e.g., leptin, insulin) acting as adiposity signals, by stomach and gut satiation signals and by hunger signals such as ghrelin (Moran and Kinzig 2004; Woods and D'Alessio 2008; Kil and Swanson 2010). The nucleus arcuatus (*NARC*), which is located in the hypothalamus, plays a key role in the processing of these signals. It includes both orexigenic (appetite-stimulating) neurons expressing Neuropeptid Y *(NPY*) and Agouti-related Peptid (*AgRP*) and anorexigenic (appetite-inhibiting) neurons

expressing Proopio-Melanocortin *(POMC*), Cocain- and Amphetamine-regulated Transcript (*CART*) (Schwartz et al. 2000). These types of neurons are sensitive to the actions of both insulin and leptin (Woods and D'Alessio 2008). Leptin, secreted from adipose tissue proportionally to body fat mass, modulates the anabolic and metabolic regulating cycles in the brain (Martin et al. 2001; Kanchuk et al. 2002). These regulating cycles are controlled by peripheral satiation and hunger signals such as cholecystokinin (*CCK*), glucagon-like peptide-1 (*GLP-1*), peptide tyrosine-tyrosine (*PYY*) and ghrelin. Leptin modulates the regulating cycles by increasing the sensitivity of the hypothalamus to satiation signals (Woods and D'Alessio 2008). Satiation is described as the increasing sensation of fullness during a meal. Satiety, in contrast, is the motivation to not eat between meals (Backus 2006). Leptin concentration is positively correlated to the body fat percentage of an individual, and it reflects the actual energy status (Suzuki et al. 2012). High body fat mass is notoriously correlated to an impaired tissue sensitivity to the action of insulin, which is commonly referred as impaired peripheral insulin sensitivity (Kolterman et al. 1980; Appleton et al. 2001). Leptin resistance also characterizes the obese state. In obese individuals, despite having higher leptin concentrations than lean individuals do, this condition causes the appetite to not be effectively suppressed (Crujeiras et al. 2015). Appleton et al. (2002) demonstrated that increased plasma leptin concentrations are associated with decreased insulin sensitivity in cats, independent of adiposity. Various abnormalities can occur in the complex energy homeostasis mechanisms, and these abnormalities are involved in the development of obesity. Studies in humans have demonstrated that polymorphism in the CCK-1 receptor gene and the PYY allele are associated with increased body weight (Funakoshi et al. 2000; le Roux et al. 2006). In the cat breeding family of the IAN, segregation analysis led to the detection of a genetic contribution in overweight animals. The genotyping analysis identified markers in the regions of the chromosome D3 containing melanocortin receptor 4 (*MC4R*) and neuropeptide Y receptor 1 (*NPY1R*) as being associated with the body condition score (Wichert et al. 2012a). Both receptors are located in the *NARC* and are known to be involved in the regulation of food intake and energy expenditure. The receptors have also been associated with overweight in humans (Woods and D'Alessio 2008). Haring et al. (2012), observed a negative correlation between overweight and insulin sensitivity. Measurements for this study were performed at the age of 8 months, when the cats were already overweight (Haring et al. 2012). In humans, a mutation at the *MC4R* locus has an increasing influence on the body mass index (BMI), starting at 8 years of age (Warrington et al. 2015). Wichert et al. (2012) observed a significantly higher food intake by adult male cats with a predisposition to overweight compared to that of cats without this predisposition.

The aim of the present study was to quantify the food intake behavior and energy expenditure in growing kittens to understand the mechanisms involved in the development of obesity early in life. Our hypothesis was that a predisposition to overweight is correlated to a higher food intake and lower energy expenditure early in life. A second hypothesis was that insulin sensitivity and circulating leptin are correlated only with the body fat percentage in the investigated cat family and are not altered prior to the development of overweight.

Material and methods

Animals and study design

A total of 26 intact kittens (11 females, 15 males) from ten litters from the breeding family of the IAN with a mean birth weight of 101.1 g (SE ± 2.5) were investigated. The litter size ranged from 1 to 7 kittens. All kittens were kept under the conditions specified in the study design and were clinically healthy. An ethical approval for animal experimentation was obtained from the Cantonal Veterinary Office of Zurich (license number 128/2014).

All kittens of each litter lived with their mothers and were nursed for the first 8 weeks of age. Additionally, the kittens were fed with a moist commercial dry food (dry matter (DM): 94%, crude protein (CP): 38%, crude fat (CL): 25.5%, crude fiber (CF): 1.2%, crude ash (CA): 6.8%, metabolizable energy (ME): 1.89 MJ/100 g) and with mushed wet food (DM: 20%, CP: 8%, CL: 5%, CF: 1%, CA: 2.5%, ME: 0.36 MJ/100 g) ad libitum from the age of three weeks and were fully weaned at 8 weeks of age. During the experiment, a different commercial dry food (Swiss Professional Kitten Chicken, Biomill SA, Herzogenbuchsee, Switzerland) was fed from the 17th week of age until the age of 8 months (DM: 95%, CP: 34%, CL: 18%, CF: 2%, CA: 7%, ME: 1.73 MJ/100g), ad libitum. Estimations of ME were calculated according to Kienzle et al. (1998). The kittens were weighed daily for the first 2 weeks of life, every second day until weaning and once per week until 8 months of age. The body condition score (BCS) of each cat was evaluated monthly from the age of 2 until 8 months of age. Energy expenditure was measured for five days and food intake for 14 days at the ages of 4 and 6 months. Dual-energy X-ray absorptiometry (DEXA) was performed and blood samples were drawn at the ages of 4, 6 and 8 months (Figure 1).

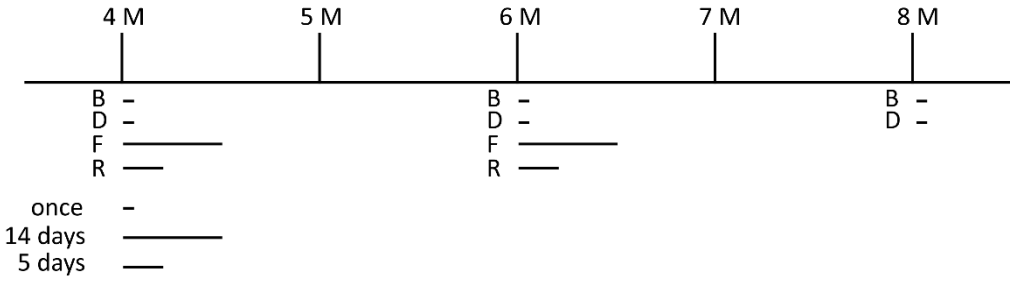

Figure 1. Experimental study design, where M=months (age), D=dual-energy X-ray absorptiometry, F=food intake (14 days), R=respiration chambers (5 days), and B=blood sampling. Additionally, body weight was measured monthly, and daily during the respiration chamber phase. Body condition score was assessed monthly, starting at 2 months of age.

Body fat percentage

DEXA was performed at the ages of 4, 6 and 8 months. Sedation was achieved with 50 µg/kg medetomidine i.m. (Dorbene ® ad us. vet., solution for injection) and 50 µg/kg butorphanol i.m. (Morphasol-4 ad us. vet., solution for injection). The cats were scanned in sternal recumbency for 204 seconds using the infant-type body scan (Hologic QD A 85508 discovery machine and QDR APEX system software version 3.3, 35 Crosby Drive, Bedford, Ma, USA).

Body condition score

The BCS of each cat was evaluated monthly from the age of 2 until 8 months according to a scale from 1 to 9 defined by Laflamme (1997), that was modified to match the physiological differences between young kittens and adult cats, which included eliminating the emphasis on the rounding of the abdomen. The palpability of the ribs and their fat covering were considered in two separate points (Table 1). Cats were classified as ideal with a BCS of 5, as overweight starting from a BCS of 6, and as obese from a BCS of 8, as in the study by Laflamme (1997).

Table 1. Body Condition Score scoring sheet after Laflamme (1997), modified to match the physiological differences in young kittens compared to adult cats.

BCS	Ribs palpability	Ribs fat covering	Waist visibility	Abdominal fat pad	Lumbar vertebrae and wing of ilia palpability and fat covering
1	Visible, very easily palpable	No palpable fat, muscle atrophy	Very exposed	None	Visible and very easily palpable, no fat
2	Between visible and easily palpable	No palpable fat, minimal muscle mass	Between very exposed and obvious	Between none and scarce	Visible, between very easily and easily palpable, no fat
3	Easily palpable	Minimal	Obvious	Scarce	Visible, easily palpable no fat
4	Between easily palpable and palpable	Between minimal and slight	Between obvious and visible	Between scarce and minimal	Barely visible, between easily palpable and palpable, between no fat and minimal fat covering
5	Palpable	Slight	Visible	Minimal	Palpable, Minimal fat covering
6	Between palpable and not easily palpable	Between slight and moderate	Barely visible	Between minimal and moderate	Moderately palpable, moderate fat covering
7	Not easily palpable	Moderate fat cover	Poorly discernable	Moderate	Hardly palpable, obvious fat covering
8	Between not easily and not at all palpable	Between moderate and heavy	Between poorly discernible and absent	Between moderate and extensive	Very heavy fat covering
9	Not at all palpable	Heavy	Absent	Extensive	Not palpable, heavy deposit of fat

Energy expenditure

Energy expenditure was measured using indirect calorimetry in two open-circuit respiration chambers at the Metabolic Centre of the University of Zurich and the Swiss Federal Institute of Technology, Agrovet-Strickhof, Lindau. One kitten per chamber (150 cm x 100 cm x 93 cm) was measured for four days, after an adaptation phase of one day. Toys, a scratcher, water and the aforementioned commercial dry food were provided to facilitate normal cat behavior. A specially designed cat toilet (Schade 2006) containing non-absorbing polypropylene cat litter permitted the collection of urine and feces totally and separately on a daily basis and included a cooling system for the urine. During the respiration measurements, food intake was measured

daily. The measuring periods lasted 22.5 hours, from 10:30 am to 9:00 am of the next day. From 9:00 am to 10:30 am, the respiration chambers were opened and cleaned, urine and feces collected, food weighed and refilled, and water refreshed. While these tasks were performed, the kittens were weighed and given free time together in a separate room under supervision. During the measuring periods, the produced volumes of O_2, CO_2 and CH_4 were measured with a gas analyzer (Promethionin, GA-4, Sable Systems Europe GmbH, Berlin, Germany), with airflows set to 60 L/min (Promethion FG-1000 and FG-250 flow generators, Sable Systems). In the chambers, a temperature of 22 ± 1°C. a relative humidity of 55% and an air pressure of approximately -60 Pa were maintained. Calibration of the gas analyzers was performed automatically before each measurement using pure N_2 (99.999%) and a mixed calibration gas (0.5% CO_2 and 0.1% CH_4 in N_2 as a carrier). Recovery of the instrument was tested before each experimental period by burning propane gas. The mean recovery rate of O_2 and CO_2 from the two chambers was 96.9% and 94%, respectively. In both the respiration chambers and in the group housing, the daily light exposure was set to summer levels (6 am to 22 pm). O_2 consumption and CO_2 production were determined with the body weight, metabolic body weight ($BW^{0.67}$) and fat-free mass (BW_{ff}). Dried food and lyophilized urine and feces were analyzed for gross energy using an anisothermal bomb calorimeter (IKA calorimeter C2000 Basic, IKA-Werke GmbH, Staufen, Germany). Liquid, defrosted urine at room temperature and lyophilized feces and dried food were analyzed for nitrogen (N) and carbon (C) using a CN analyzer (TruMac CN, Leco Corporation, St. Joseph, MI, USA). The dry matter and ash contents were analyzed at the same time using a thermogravimetric analyzer (TGA-701, Leco Corporation). The crude fat and crude fiber contents of the food samples were analyzed following the proximate analysis described by Naumann K. (2004). Crude protein was calculated by multiplying the N content by 16, based on the assumption that proteins have a mean N content of 16%. Nitrogen-free extract amounts were calculated using the following formula: NfE = DM – (CA + CP + CL + CF), where DM = dry matter, CP = crude protein, CL = crude fat, and CF = crude fiber. Nitrogen, carbon and energy balances were calculated following the formulas of Brouwer (1965) with reference to both metabolic body weight and fat-free mass (formulas only shown for $BW^{0.67}$):

Retained nitrogen: $N_{retained} = N_{food} - (N_{faeces} + N_{urine})$, all in $\frac{g}{kg\ BW^{0.67} x\ d}$

Retained carbon: $C_{retained}$

$$= C_{food} - (C_{faeces} + C_{urine} + C_{CO_2} + C_{CH_4}), \text{ all in } \frac{g}{kg\ BW^{0.67}\ x\ d}$$

There is only a negligible or no production at all of methane (CH_4) in the feline species (Schade 2006).

$$\text{Retained energy after the CN method: } E_{ret_{CN}}\left(\frac{kJ}{g\ BW^{0.67} x\ d}\right)$$

$$= 51.83 * C_{retained}\left(\frac{g}{kg\ BW^{0.67} x\ d}\right) - 19.40 * N_{retained}\left(\frac{g}{kg BW^{0.67} x\ d}\right)$$

$$\text{Deposition of protein: } D_{protein} = 6.25 * N_{retained}, \text{ all in } \frac{g}{kg\ BW^{0.67}\ x\ d}$$

$$\text{Fat gain } \left(\frac{g}{kg\ BW^{0.67} x\ d}\right) = \frac{\left(E_{retained} - D_{protein}\right) * 23.8\ kJ/g}{39.7\ kJ/g}$$

$$\text{Metabolisable energy: ME}$$

$$= gross\ energy_{food} - gross\ energy_{faeces} - gross\ energy_{urine}$$

$$- gross\ energy_{CH_4}, \text{ all in } \left(\frac{kJ}{kg\ BW^{0.67}\ x\ d}\right)$$

$$\text{Metabolizability of energy} = \frac{ME}{gross\ energy_{food}}, \text{ all in } \frac{kJ}{kg\ BW^{0.67} x\ d}$$

After Christensen et al. (1988),

$$\text{Energy Expenditure: EE = Heat Production (Q) = ME - } E_{ret_{CN}}, \text{ all in } \frac{kJ}{kg\ BW^{0.67} x\ d}$$

The means and standard errors (SE) were also calculated.

Food intake

Food intake behavior was measured for 14 days at 4 and 6 months of age using an automatic feeding station (Wichert et al. 2012). From the data registered by the feeding station, the following metrics were calculated: food intake per day, number of meals per day, meals during daytime (6 am-22 pm), meals during darkness (22 pm – 6 am), time between meals, meal length, and food intake per meal. The intake of metabolizable energy (ME) was calculated using the ME content of the dry food, as determined during the respiration measurement (Wichert et al. 2012).

Blood values

Blood samples were collected from the *vena cephalica* after four hours of fasting at 4, 6 and 8 months of age and were analyzed immediately for glucose content with a validated blood glucose meter (Ascencia Elite^{TM}, Bayer Inc. Healthcare Division, Toronto, Canada) (Wess and Reusch 2000). After centrifugation, plasma was stored at -80°C and subsequently analyzed for insulin and leptin concentration. Insulin concentration was measured by a feline-specific enzyme radioimmunoassay (Mercodia Feline Insulin ELISA, Mercodia, Uppsala, Sweden), validated for use in cats by Strage et al. (2012). In this study, they report intra- and interassay coefficients of 2.0-4.2% and 7.6-14%, respectively. Leptin concentration was determined using a multi-species leptin radioimmunoassay (Multi-species Leptin RIA Kit XL-85K, Millipore, Missouri, USA). Backus et al. (2000) validated this test kit for use in cats and found an intra- and interassay coefficients of 2.8-3.6% and 6.5-8.7%, respectively.
The insulin sensitivity index was calculated following the homeostasis model assessment (HOMA) (Matthews et al. 1985) which was developed to measure human insulin sensitivity:

$$\frac{Insulin\ \left(\mu U/_{ml}\right) * Glucose\ (mmol/_{l})}{22.5}$$

This index was validated for use in cats by Appleton et al. (2005).

Statistical analysis

Statistical analysis was performed using Excel (Office Excel Professional Plus 2013, Microsoft Corporation, Redmond, WA, USA) and R version 3.3.1. (R Core Team 2016). The dependence of the outcomes on various factors was tested using the R function for generalized linear models (GLM) (family="Gaussian"), with the models following the form of outcome~factor1+ factor2+ factor1*factor2. The results of BCS and body fat percentage at 8 months were tested for their dependence on the factors of sex, food intake, energy expenditure, fat gain, protein deposition, blood leptin concentration, and food intake behaviors at 4 and 6 months of age (meals per day, grams per meal, grams per minute, duration of the meal). The association of BCS with body fat percentage at 4, 6, and 8 months of age was also tested. Further, the association of body fat percentage and insulin sensitivity was assessed. The association of food intake with body fat percentage and blood leptin concentration, food intake behaviors, and sex as well as the association of food intake behaviors at 8 months of age and food intake at 4 and 6 months were also assessed. For all models, interactions between the same factors at two different time points (4 and 6 months of age) were considered. The threshold for statistical

significance was set at $p<0.05$. Only factors with a significant impact on the outcomes are reported. The median values of the aforementioned factors were also calculated.

Results

Body fat percentage

Median with the minimum and maximal values for all analysed factors are listed in Table 2. Body fat percentage at 8 months was significantly associated with food intake (with reference to both $BW^{0.67}$ and BW_{ff}), food intake rate, blood leptin concentration and male sex (details in Table 3 and Supplementary Table 1). Energy expenditure at 4 and at 6 months alone had no association with body fat percentage at 8 months of age. Body fat percentage was not correlated to the insulin sensitivity index at 8 months of age. Median with the minimum and maximal values for insulin sensitivity index are listed in Table 2.

Table 2. Median values (min-max) of the analysed factors Body Condition Scores, body fat percentage, food intake, energy expenditure, fat gain, protein deposition, blood serum leptin concentration, meals per day, meal size, food intake rate, duration of meal and insulin sensitivity index (n=22 at 4 months, and n=26 at 6 months and 8 months, if available).

Factor	Units	4 months	6 months	8 months
Body Condition Score	1-9	5.3 (4.8-6.0)	5.5 (4.8-6.8)	5.7 (4.9-6.7)
Body fat percentage	%	12.0 (5.7-19.7)	13.9 (6.8-25.1)	14.0 (6.8-22.3)
Food intake	$\frac{g\ DM}{kg\ BW^{0.67}\ x\ d}$	52.5 (35.0-76.4)	39.6 (27.0-50.7)	
Energy expenditure	$\frac{kJ}{kg\ BW^{0.67}\ x\ d}$	433.7 (293.0-587.5)	455.5 (344.7-563.0)	
Fat gain	$\frac{g}{kg\ BW^{0.67} x\ d}$	8.1 (2.0-14.0)	4.7 (-1.0-10)	
Protein deposition	$\frac{g}{kg\ BW^{0.67}\ x\ d}$	6.3 (3.0-11.0)	4.3 (1.0-9.0)	
Blood serum leptin concentration	$\frac{ng}{ml}$	15.9 (4.8-33.9)	16.8 (4.3-50.7)	17.5 (6.0-35.5)
Meals per day		7.2(3.7-12.1)	8.2 (4.3-13.8)	
Meal size	$\frac{g\ DM}{meal}$	14.8 (4.6-27.3)	12.4 (4.2-23.0)	
Food intake rate	$\frac{g\ DM}{min\ of\ stay}$	2.5 (1.3-3.8)	2.5 (1.3-3.9)	
Duration of meal	min	6.8 (3.1-12.6)	5.2 (2.5-10.2)	
Insulin Sensitivity Index		0.5 (0.1-1.3)	0.5 (0.1-2.3)	0.6 (0.2-1.9)

Table 3. Factors food intake at 4 months, food intake rate at 4 and 6 months, blood serum leptin concentration at 8 months and sex associated with body fat percentage at 8 months in a generalized linear model (n=22 at 4 months, and n=26 at 6 months and 8 months).

Factor	Units	Estimate (β)	S.E.	p
Food intake at 4 months	$\frac{g\ DM}{kg\ BW^{0.67}\ x\ d}$	2.52	0.96	0.02
Food intake rate at 4 months	$\frac{g\ DM}{\min\ of\ stay}$	2.75	0.82	0.004
Food intake rate at 6 months	$\frac{g\ DM}{\min\ of\ stay}$	2.64	0.81	0.005
Blood serum leptin concentration at 8 months	$\frac{ng}{ml}$	0.37	0.15	0.02
Sex: male		-0.1	1.47	<0.001

Body Condition Score

The distributions of the BCS are illustrated in Figure 2. BCS at 8 months of age was found to be significantly associated with the food intake (with reference to $BW^{0.67}$ and BW_{ff}) at 6 months, food intake rate (grams per minute of stay per cat) at 4 and 6 months, blood leptin concentration, and the interaction of energy expenditure and food intake at 6 months. The energy expenditure at 4 and at 6 months alone had no association with BCS at 8 months of age. Males had a significantly lower BCS than females did. Food intake and energy expenditure relating to metabolic BW at 6 months were negatively correlated (details in Table 4). However, no association was found for BW_{ff} (see Supplementary Table 2). BCS and body fat percentage were significantly associated at every measured time point (see Table 5).

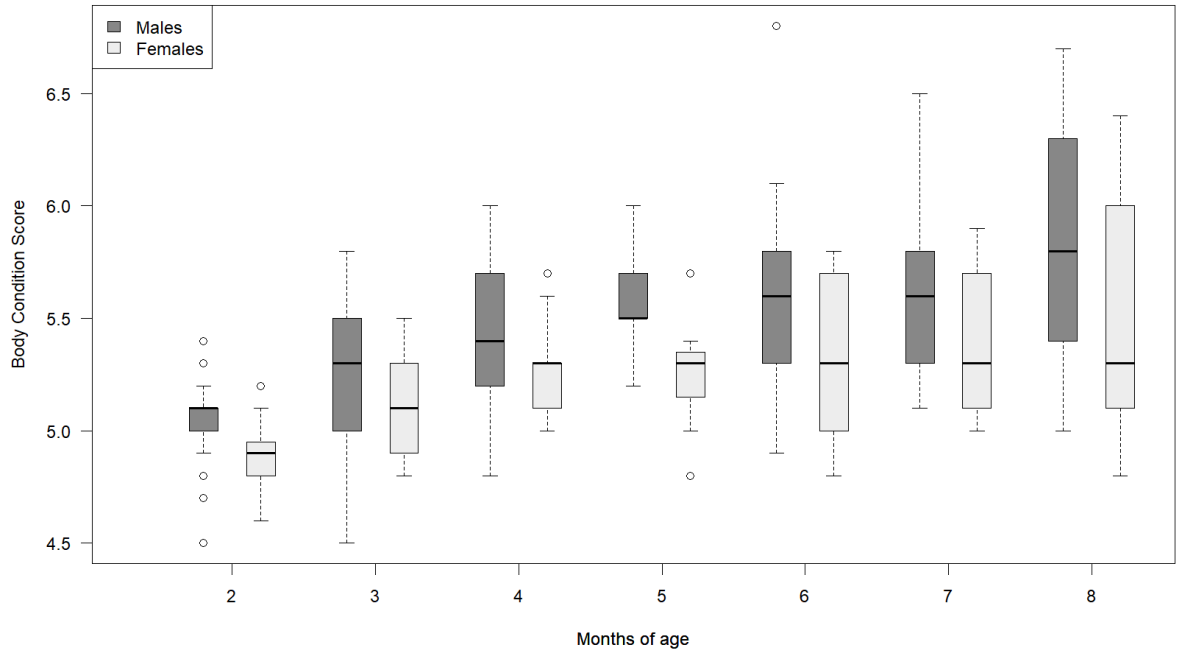

Figure 2. Box plots of monthly Body Condition Score distributions between 2 and 8 months of age (females=11, males=15).

Table 4. Factors food intake at 6 months, food intake rate at 4 and 6 months, blood serum leptin concentration at 8 months, sex and food intake at 6 months with energy expenditure at 6 months associated with Body Condition Score at 8 months in a generalized linear model (n=22 at 4 months, and n=26 at 6 months and 8 months).

Factor	Units	Estimate (β)	S.E.	p
Food intake at 6 months	$\frac{g\ DM}{kg\ BW^{0.67}\ x\ d}$	0.31	0.08	0.004
Food intake rate at 4 months	$\frac{g\ DM}{min\ of\ stay}$	0.27	0.09	0.01
Food intake rate at 6 months	$\frac{g\ DM}{min\ of\ stay}$	0.26	0.09	0.01
Blood serum leptin concentration at 8 months	$\frac{ng}{ml}$	0.04	0.02	0.049
Sex:male		-0.47	0.14	0.006
Food intake at 6 months: Energy expenditure at 6 months	$\frac{\frac{g\ DM}{kg\ BW^{0.67}\ x\ d}}{\frac{kJ}{kg\ BW^{0.67}\ x\ d}}$	-0.0004	0.0001	0.005

Table 5. Association between Body Condition Score (BCS) and body fat percentage in a generalized linear model (n=22 at 4 months, and n=26 at 6 and 8 months).

Association	Estimate (β)	S.E.	p
BCS and body fat content at 4 months	0.047	0.016	0.009
BCS and body fat content at 6 months	0.043	0.015	0.01
BCS and body fat content at 8 months	0.086	0.018	0.0002

Food intake

Food intake at 6 months of age (with reference to both $BW^{0.67}$ and BW_{ff}) was associated with meal frequency and size, along with male sex (details in Table 6 and Supplementary Table 3). Food intake at 4 months of age (with reference to $BW^{0.67}$) was associated with meal frequency and size and was negatively associated with food intake rate (details in Table 6).

Table 6. Factors meal frequency at 6 months, meal size at 6 months, sex, meal frequency at 4 months meal size at 4 months and food intake rate at 4 months associated with food intake at 6 months (n=26, indicated with superscript letter A), and 4 months (n=22, indicated with superscript letter B).

Factor	Units	Estimate (β)	S.E.	p
Meal frequency at 6 months[A]	$\frac{meals}{d}$	7.01	1.67	0.001
Meal size at 6 months[A]	$\frac{g\ DM}{meal}$	3.13	0.74	0.001
Gender: male[A]		8.83	2.63	0.003
Meal frequency at 4 months[B]	$\frac{meals}{d}$	9.17	3.51	0.025
Meal size at 4 months[B]	$\frac{g\ DM}{meal}$	4.83	1.13	0.001
Food intake rate at 4 months[B]	$\frac{g\ DM}{\min of\ stay}$	-21.7	8.88	0.031

Discussion

In this study, kittens that became overweight ate more and faster than their lean littermates did. This was shown by the association of both the daily food intake and food intake rate with the development of overweight. Both food intake and food intake rate were positively associated with a higher BCS and body fat percentage at 8 months of age. In humans, it has been extensively demonstrated that quick eaters have a significantly higher body mass index (BMI) and body weight (Christensen et al. 1988) than slow eaters do. Among the possible explanations for this phenomenon, it can be assumed that by eating faster, more energy is ingested before the satiation signals are recognized by the brain, which are triggered on nutrient ingestion by gastric distension and the release of gut factors, including CKK (Morton et al. 2006). Another important finding is that kittens who ate more overall had larger meals at a higher frequency at

6 months of age. These findings together may indicate a possible disorder in the satiation and satiety feedback in cats that develop an overweight phenotype early in life (Haring et al. 2011). Similarly, in adult humans, eating frequency (both meals and snacks) has been positively associated with overweight/obesity (Murakami and Livingstone 2015), and an earlier study by the same authors also showed that a higher eating frequency was associated with a higher BMI in adolescents and children (Murakami and Livingstone 2014). A higher food intake per meal and an increased meal frequency can lead to a positive energy balance and, therefore, to overweight (Fettman et al. 1997). In Labrador and Flat-Coated Retrievers, Raffan et al. (2016) found a deletion in the *POMC* gene that was associated with increased body weight, adiposity, and food motivation. The adult intact overweight male cats from the cat family examined in this study, however, were previously found to have a significantly higher food intake rate and an increased meal size but no increased meal frequency (Wichert et al. (2012). The differing eating behavior of these cats could have been influenced by the weight-loss program they underwent before the experiment, which could have resulted in atrophy of the existing adipocytes and led to important adaptations in energy homeostasis mechanisms (MacLean et al. 2015). This phenomenon, also referred as "weight-cycling" or "yo-yo dieting" is well known, both in human beings (Blackburn et al. 1989; Brownell 1989) and in animals such as dogs and cats (Villaverde et al. 2008; Nagaoka et al. 2010; MacLean et al. 2015).

At six months of age, cats with an increased food intake had lower total energy expenditures. The main components of total energy expenditure are resting energy expenditure, energy expenditure for the processing of ingested food, and activity-related energy expenditure. These factors are mainly determined by body size, food intake, physical activity and body composition. Body fat has a five-fold lower contribution to resting energy expenditure compared to that of fat-free mass, ~3.5 kcal/kg/d vs. ~18 kcal/kg/d, respectively (Wang et al. 2000; Browning and Evans 2015). In the present study, kittens with a higher BCS also had a higher body fat percentage. Increased food intake was additionally correlated to a higher body fat percentage, which could have contributed to a relatively lower energy expenditure. When analyzed with reference to BW_{ff}, no significant association could be determined between food intake and energy expenditure. The same was shown by Martin et al. (2001), who described a significantly lower energy expenditure in adult cats after gonadectomy on a bodyweight basis; however, as in the present study, these results were not significant when evaluated on a BW_{ff} basis. In our study, no significant correlation was found between energy expenditure at 6 months and BCS or body fat percentage at 8 months. Activity-related energy expenditure also

impacts total energy expenditure (Westerterp 2017), and a low activity level is one of the most common causes of overweight in both humans (Pengpid and Peltzer 2016; Trandafir and Temneanu 2016) and cats (Scarlett et al. 1994; Colliard et al. 2009). However, we did not measure the activity level in the present study. Thus, it would be interesting to quantify the activity of growing and adult cats with and without a predisposition to overweight.

Our initial hypothesis that a predisposition to overweight is connected to a higher food intake and lower energy expenditure early in life is partially wrong because a higher food intake was found to be positively correlated to overweight, whereas energy expenditure was not. Considering these results, it can be assumed that cats with a predisposition to overweight have an altered regulation of food intake with an unaltered energy metabolism.

At all measured time points, there was no correlation between fat gain (measured with help of carbon balances) and BCS or body fat percentage (data not shown). This could be due to the different durations and time points of the two measurements (respiration chambers were measured for five days, whereas food intake measurements were taken for 14 days). As all animals present a certain fat accretion during growth (Dobenecker et al. 2013), it is possible that the difference in fat gain measured during the short period in the respiration chambers was too modest to be significant.

BCS is a subjective assessment and therefore has its limitations, especially since a modified version for growing kittens was used. However, BCS and body fat percentage were correlated at all measured time points. The estimate was lower at 4 and 6 months compared to 8 months old. This could be due to the smaller size and weight of the kittens at 4 and at 6 months of age, which was at the lower limit of Software of the infant body scan. In order to use the same device for all three measurements, the use of a DEXA scan designed for rats was not considered. Overall, in our study, male kittens had a lower BCS and body fat percentage but a higher food intake compared to their female counterparts. The higher body fat percentage in females is in agreement with the findings of other studies in cats (Martin et al. 2001) and in pubertal and adult humans (Wells 2007; Loomba-Albrecht and Styne 2009).

In the present study, no association between leptin levels and food intake at 4 or 6 months of age could be determined. Physiologically, leptin is known to decrease food intake by suppressing the appetite and to be secreted proportionally to the body fat percentage (Woods and D'Alessio 2008). However, the leptin blood levels at 8 months of age were found to be positively correlated to an increased BCS and body fat percentage at the same age. These results

are in agreement with those of previous studies (Martin et al. (2001) and Backus et al. (2000)). The male kittens in our study had no significant difference in leptin blood concentration compared to the females, which is in contrast to findings in humans (Koester-Weber et al. 2014). In human adolescents, it is known that there is a noteworthy variability of metabolic parameters, including leptin, between sexes (Cook et al. 2003; Weiss and Caprio 2005). The mean age at the onset of puberty in domestic shorthair cats is between 4.5 and 15 months (Peterson 2010), and its onset is dependent on the body weight (Scott 1971; Lein and Concannon 1983) and season of the year (Hurni 1981). In the present study, the examined kittens were born in different seasons of the year, so we cannot exclude that they entered puberty at different time points within the measured time frame of 6 months, which could have led to an increase in the variability of leptin blood concentration.
In humans, the activity level also has an influence on leptin blood values, as described by Jimenez-Pavon et al. (2012), which emphasises the need for further investigating the activity level of overweight cats.

No significant correlation between BCS, body fat percentage and insulin sensitivity was found in our growing kittens, which is in agreement with other studies conducted with adolescent cats (Rigamonti et al. 2013). In the study by Rigamonti et al. (2013), obese adults were significantly more insulin-resistant than lean adults were. This condition could not be proven in obese adolescents. In adolescents, it is known that there is a noteworthy variability of metabolic parameters, including insulin (Cook et al. 2003; Weiss and Caprio 2005). Consequently, our second hypothesis that insulin sensitivity and leptin blood concentration are correlated only to the body fat percentage and are not altered prior to the development of overweight was confirmed. HOMA analysis was used to calculate the insulin sensitivity index because of its lower invasiveness compared to that of the minimal model estimation of insulin sensitivity or a glucose tolerance test, according to Appleton et al. (2005), which validated its use in cats. However, we cannot exclude that assessment of insulin sensitivity with the aforementioned measuring techniques could have led to other results.

Conclusion

It was shown that kittens developing a higher BCS at an early age had a higher food intake but the same energy expenditure compared to those of lean kittens. These findings indicate a possible disorder in the satiation and satiety feedback in cats which develop an overweight phenotype early in life. In the future, the focus of further studies could include investigating the

variability at genetic loci connected to food intake (*MC4R, NPY1R, POMC*) and the activity level of predisposed cats.

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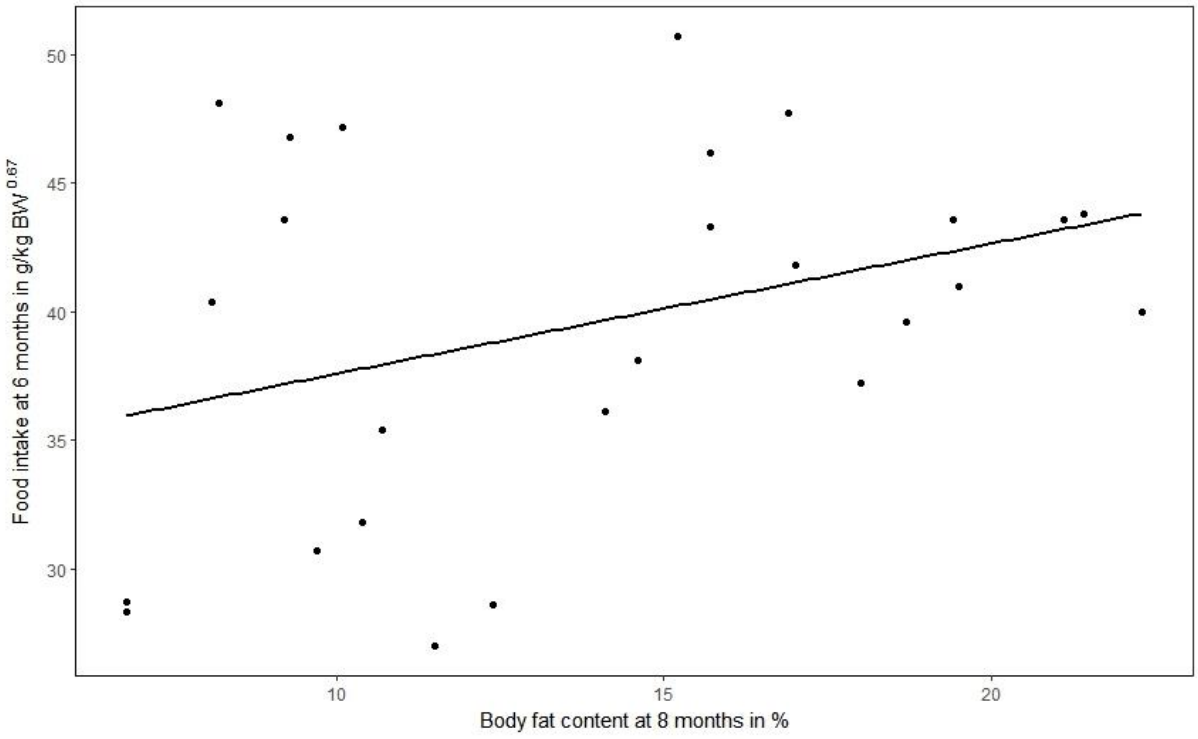
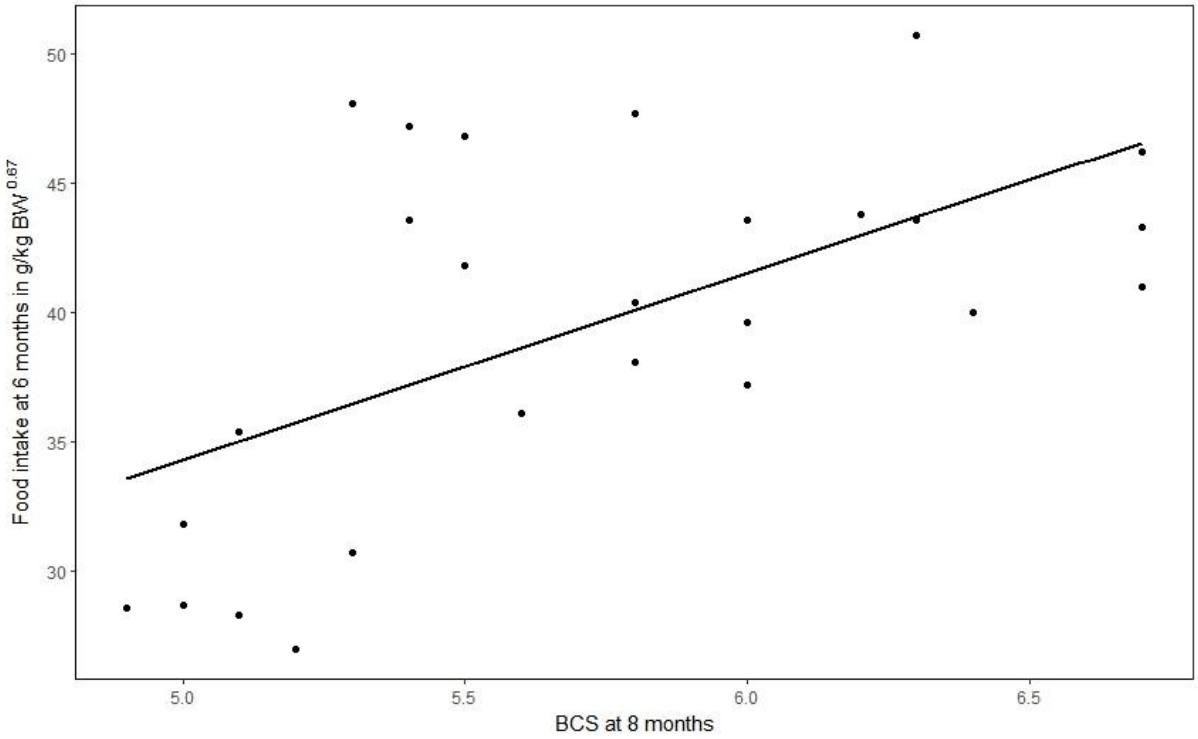
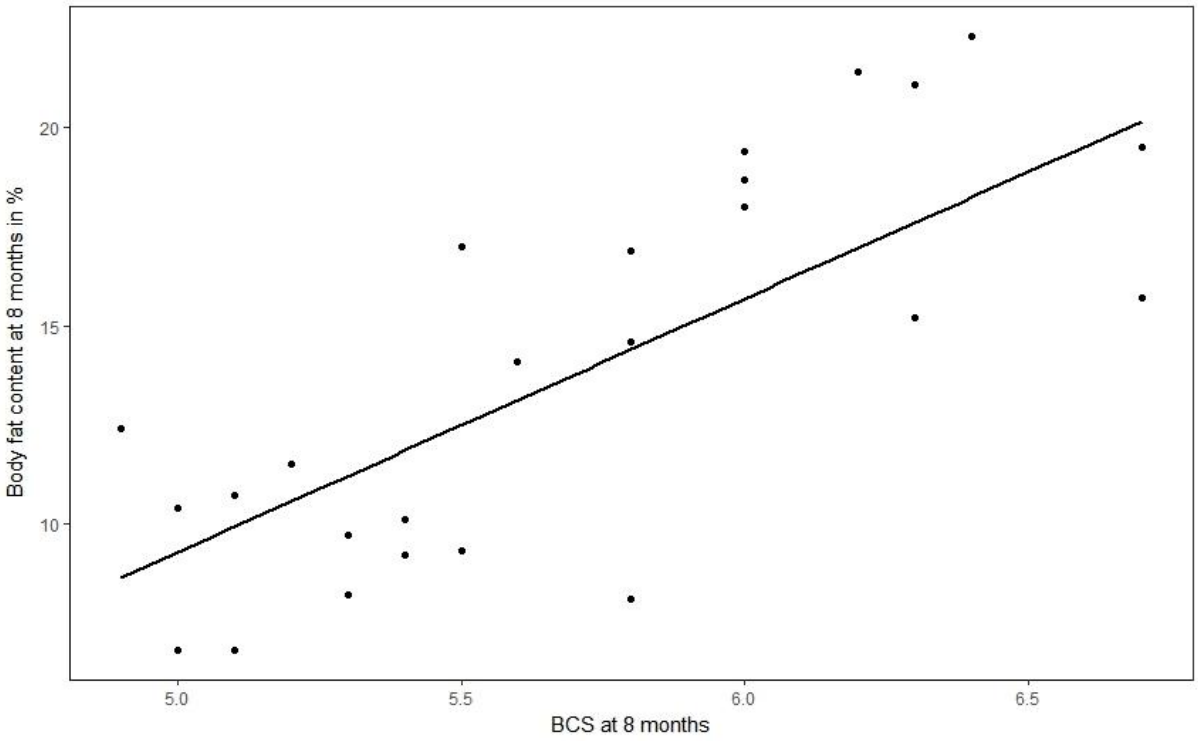
Anhänge



Supplementary Figure 1. Relationship between food intake/kg $BW^{0.67}$ at six months and body fat percentage at eight months (n=26).



Supplementary Figure 2. Relationship between food intake/kg $BW^{0.67}$ at six months and Body Condition Score at eight months (n=26).



Supplementary Figure 3. Relationship between body fat percentage and Body Condition Score at eight months (n=26).

Supplementary Table 1. Factors food intake at 4 and 6 months, food intake rate at 4 and 6 months, blood serum leptin concentration at 8 months and sex associated with body fat percentage at 8 months in a generalized linear model (referred to BW_{ff}, n=22 at 4 months, and n=26 at 6 and 8 months).

Factor	Estimate (β)	S.E.	p
Food intake at 4 months (g DM/kg BW_{ff}/d	1.87	0.71	0.02
Food intake at 6 months (g DM/kg BW_{ff}/d	2.51	0.94	0.02
Food intake rate at 4 months (g DM/min of stay)	2.75	0.82	0.004
Food intake rate at 6 months (g DM/min of stay)	2.64	0.81	0.005
Food intake rate at 4 months: Food intake rate at 6 months (g DM/min of stay)	-0.13	0.04	0.01
Blood serum leptin concentration at 8 months (ng/ml)	0.37	0.15	0.02
Sex: male	-0.1	1.47	<0.001

Supplementary Table 2. Factors food intake at 6 months, energy expenditure at 4 and at 6 months, food intake rate at 4 and 6 months, blood serum leptin concentration at 8 month and sex associated with Body Condition Score at 8 months in a generalized linear model (referred to BW_{ff}, n=22 at 4 months, and n=26 at 6 and 8 months).

Factor	Estimate (β)	S.E.	p
Food intake at 6 months (g DM/kg BW_{ff}/d	0.32	0.12	0.02
Energy expenditure at 4: Energy expenditure at 6 months (kJ/kg BW_{ff}/d)	0.00005	0.00002	0.04
Food intake rate at 4 months (g DM/min of stay)	0.27	0.09	0.01
Food intake rate at 6 months (g DM/min of stay)	0.26	0.09	0.01
Blood serum leptin concentration at 8 months (ng/ml)	0.04	0.02	0.049
Sex: male	-0.47	0.14	0.006

Supplementary Table 1. Factors meal frequency at 6 months, meal size at 4 and 6 months, duration of meal at 4 months and sex associated with food intake at 6 months (referred to BW_{ff}, n=22 at 4 months, indicated with superscript letter A, and n=26 at 6 months, indicated with superscript letter B).

Factor	Estimate (β)	S.E.	p
Meal frequency at 6 months (meals/d)^{A}	5.14	1.47	0.004
Meal size at 4 months (g DM/meal)^{A}	1.62	0.53	0.011
Meal size at 6 months (g DM/meal)^{A}	2.99	0.54	0.001
Duration of the meal at 4 months (minutes/meal)	-2.42	0.89	0.020
Sex: male^{A}	-7.58	1.89	0.002
Meal size at 4 months (g DM/meal)^{B}	4.47	1.37	0.011

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